The Plant Cell, Vol. 6, 1171–1176, August 1994 © 1994 American Society of Plant Physiologists

Light Modulation of Ferredoxin mRNA Abundance Requires an Open Reading Frame

Lynn F. Dickey,[a] Thanh-Tuyen Nguyen,[a] George C. Allen,[a] and William F. Thompson [a,b,1]

[a] Department of Botany, North Carolina State University, Raleigh, North Carolina 27695
[b] Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695

Ferredoxin I (*Fed-1*) mRNA abundance is modulated by an internal light regulatory element that includes sequences both 5′ and 3′ of the translational initiation site. To test the hypothesis that the light response mediated by this element might be coupled to translation, we transformed tobacco plants with gene constructs blocked in translational initiation or elongation. Here, we report that such mutations abolish the light response in vivo. A nonsense mutation could be rescued by restoring the open reading frame with a different sequence, even when the new codon caused an amino acid substitution. Our data establish that the light response requires a translatable reading frame and thus provide strong circumstantial evidence for post-transcriptional modulation of *Fed-1* mRNA levels. The *Fed-1* system is presently the only higher plant example of a developmentally regulated change in mRNA abundance that requires translation of the affected mRNA.

INTRODUCTION

Ferredoxin I (*Fed-1*) is a nuclear gene encoding chloroplast ferredoxin in *Pisum* (Dobres et al., 1987; Dickey et al., 1992a). Like most genes encoding plastid proteins, *Fed-1* is expressed more strongly in the light than in the dark. However, its light response differs in several respects from those of other well-characterized light-responsive genes (Kaufman et al., 1986) and involves different molecular events (Thompson, 1988; Thompson and White, 1991; Neuhaus et al., 1993). Among the important differences is the fact that a major light response element in the pea *Fed-1* gene is located within the transcription unit (Elliott et al., 1989), while other light-responsive genes are regulated mainly by upstream elements (Thompson and White, 1991; Kuhlemeier, 1992). We have recently shown that the *Fed-1* internal light regulatory element (iLRE) is located within the 5′ portion of the transcribed sequence and that it includes portions of both the leader and coding region (Dickey et al., 1992b).

The location of the *Fed-1* iLRE within the transcribed portion of an intronless gene rules out transcriptional regulatory mechanisms that depend on upstream control elements and is consistent with models in which light modulates mRNA abundance by directly or indirectly affecting its rate of degradation. Direct observation of a light effect on mRNA stability has thus far been prevented by the fact that *Fed-1* mRNA is stabilized when cellular RNA synthesis is inhibited by actinomycin-D (G.C. Allen, unpublished data). However, other data provide indirect support for a post-transcriptional model. In both tobacco and Arabidopsis, changes in mRNA abundance in light-grown versus dark-adapted plants occur in the absence of corresponding changes in the transcriptional activity of isolated nuclei (Dickey et al., 1992b; Vorst et al., 1993), and the 5′ portion of a pea *Fed-1*::*GUS* (β-glucuronidase) chimeric mRNA is stabilized in the light, whereas reporter sequences in the 3′ portion of the transcript are degraded (Dickey et al., 1992b).

Recent reports indicate that the stability of an mRNA often depends on its translational activity, whereas in other cases, translation may be required for rapid mRNA turnover (for reviews, see Cleveland and Yen, 1989; Gallie, 1993; Peltz and Jacobson, 1993; Sachs, 1993; Sullivan and Green, 1993). Recent reports using polysome-based in vitro mRNA degradation systems suggest that in some of these cases, mRNA turnover may be linked to translation (Brewer and Ross, 1990; Byrne et al., 1993; Tanzer and Meagher, 1994). Because the *Fed-1* iLRE requires sequences on both sides of the translational initiation site to be fully effective (Dickey et al., 1992b), we hypothesized that the light response of this mRNA might be coupled to its translation. We tested this hypothesis by inserting missense and nonsense mutations at various positions in a chimeric *Fed-1* gene driven by the cauliflower mosaic virus (CaMV) 35S promoter. Light responsiveness of the resulting constructs was examined in vivo using transgenic tobacco plants as previously described (Dickey et al., 1992b). Our results support a post-transcriptional mechanism for iLRE-mediated light effects and provide, at present, the only higher plant example in which a regulated change in mRNA abundance has been shown to require translation of the affected mRNA.

[1] To whom correspondence should be addressed.

RESULTS

Missense and Nonsense Insertions Reduce Light Responsiveness

Figure 1 illustrates the effect of two different mutations designed to prevent translational initiation at the normal site. In the NS1 construct (Figure 1A), we inserted a TAA nonsense codon in place of the normal ATG codon. This mutation reduced the light induction ratio to 2.0 (SD = 0.5). For comparison, similar constructs with a functional initiation codon exhibit average ratios of ~4. Transformants carrying the MS1 construct (Figure 1B), in which a missense codon (ATA) was substituted for the initiation codon, also had reduced light responses; in this case induction ratios averaged 1.7-fold (SD = 0.4).

Additional results summarized in Figure 2 show that stop codons at each of several other positions near the 5′ end of the *Fed-1* open reading frame also greatly inhibit the light effect on *Fed-1* mRNA abundance. In the NS2 construct, the second codon in the *Fed-1* sequence was changed from an alanine (GCA) codon to a nonsense (TAA) codon. This mutation caused the induction ratio to decrease to ~1.2 (SD = 0.3), indicating that the light response had been virtually abolished. Nonsense mutations in codons 4 and 8 both reduce light induction below twofold (NS4, 1.5-fold, SD = 0.7; NS8, 1.7-fold, SD = 0.3), whereas the mutation designated NS25 actually has a slightly negative light response (induction ratio of 0.7, SD = 0.1). The latter mutation introduces a nonsense codon at position 25, but also changes the normal methionine codon at position 24 to ATT, which encodes isoleucine. Although the methionine codon at position 24 is one of two potential in-frame internal initiation sites in the *Fed-1* iLRE, in the presence of a functional ATG codon at position 1, it does not appear to be required for the light response. *Fed-1*::*CAT* (chloramphenicol acetyltransferase) fusions containing (FC34 construct) or lacking (FC20 construct) the ATG codons at positions 22 and 24 are similarly light responsive (Figure 2). Thus, we believe that the lack of a light response in the NS25 construct results from translational termination at position 25 rather than from the change in encoded amino acid at position 24.

Restoring the Reading Frame Restores the Light Response

To determine if mutations in the *Fed-1* open reading frame prevent light induction by blocking translation, as opposed to simply changing the RNA sequence, we tested two constructs in which the open reading frame was restored with a sequence differing from that of the wild-type message. The reference construct for these experiments was NS2, in which a nonsense codon at position 2 dramatically reduced the light response. RSF2-leu is identical to NS2 except that instead of a TAA stop codon at position 2 it contains TTA, a similar triplet that encodes leucine. Figure 3 shows that this construct is strongly

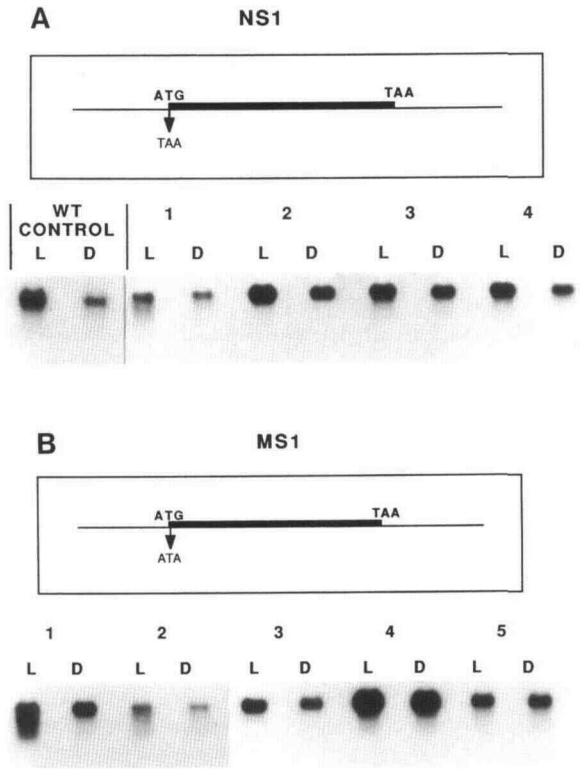

Figure 1. Gel Blots of RNA from Transgenic Plants Containing *Fed-1* Constructs with Nonsense or Missense Mutations in the Start Codon.

Constructs were derived from the *Fed-1* message construct (Elliott et al., 1989) in which the *Fed-1* transcribed sequence is fused to the CaMV 35S promoter. In the diagrams, the thicker line represents the *Fed-1* coding region; the sequence changes and approximate sites of the nonsense or missense mutations are indicated. The numbers above the gel blots designate independent primary transformants. WT control designates a representative transformant containing the wild-type message construct. Each transformant was represented by a pair of clonally duplicated transgenic tobacco plants, both of which were placed in darkness for 3 days prior to the start of the experiment. One member of each duplicate pair was then exposed to 6 hr of light immediately prior to harvest (lanes L), while the other member remained in the dark as a control (lanes D). RNA was prepared from the two youngest leaves whose blades were completely unfolded, and *Fed-1* mRNA signals were quantitated by radioanalytic imaging as described in Methods. Light induction is expressed as a ratio and represents the fold increase in *Fed-1* mRNA abundance during a 6-hr light exposure. This ratio was 4.0 for wild-type controls in this series of experiments.

(A) The NS1 construct contains a nonsense (ATG-to-TAA) mutation at the normal translation start site of *Fed-1*. The light induction ratio was 2.0 (SD = 0.5).

(B) The MS1 construct has a missense mutation (ATA, encoding isoleucine) in place of the normal translation start codon. The light induction ratio was 1.7 (SD = 0.4).

light regulated, with an average induction ratio of 3.5 (SD = 0.7). Similar results were obtained with RSF2-ala. This construct encodes alanine at the second position, as in the wild-type amino acid sequence, but uses a GCG codon rather than the wild-type GCA. In this case, we obtained a normal light response with an induction ratio of 4.3 (SD = 1.6).

The GCG codon in RSF2-ala is used infrequently in most dicot plant species, including tobacco (Wada et al., 1992), while only relatively frequent codons are used in the wild-type *Fed-1* sequence. Rare codons have been reported to destabilize several mRNAs (Peltz and Jacobson, 1993; Sachs, 1993; Sullivan and Green, 1993). However, the 4.3-fold light response of RSF2-ala is well within the range we observed for constructs containing the wild-type sequence (Figure 3D), and the abundance of RSF2-ala mRNA was not dramatically reduced relative to these controls (data not shown). Therefore, insertion of a rare codon at this position seems not to have a significant effect on *Fed-1* mRNA stability.

It is also interesting to note that the alanine codon at the second position preserves a strong initiation codon context (ATGGCG) (Gallie, 1993), whereas the leucine codon does not (ATGTTA). Thus, the slightly higher induction ratio we obtained with the RSF2-ala construct (4.3 compared to 3.5) might correlate with somewhat more efficient translation of this mRNA relative to that from the RSF2-leu construct.

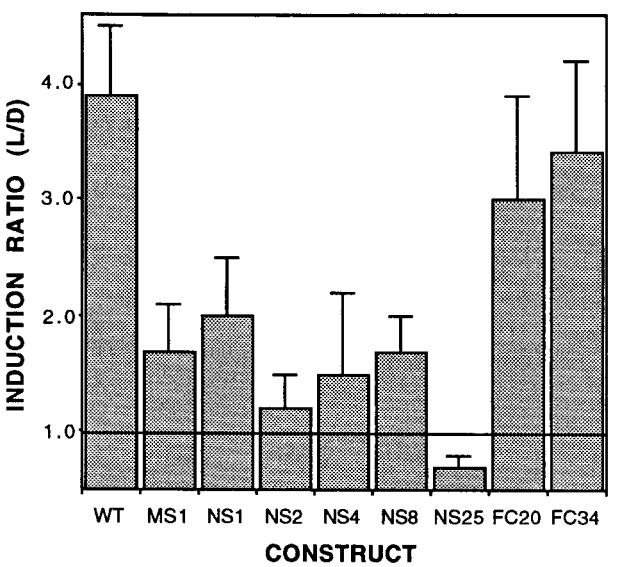

Figure 2. Results Obtained with Constructs Containing Mutations in Various Codons.

The bar labeled WT represents the light response observed with the wild-type message construct from which all the other constructs were derived. The missense construct, MS1, is described in Figure 1. NS constructs were derived from the 35S::*Fed-1* message construct (Elliott et al., 1989) by substituting a nonsense codon (TAA) in place of the wild-type codon at the indicated position. The nonsense codon in NS2 replaces the alanine codon at position 2, that in NS4 replaces a threonine codon at position 4, and that in NS8 replaces a tyrosine codon at position 8. The NS25 mutation inserts a nonsense codon in place of the serine codon at position 25 and changes the methionine codon at position 24 to isoleucine (ATT). FC20 and FC34 are translational fusions with the *CAT* gene (obtained from pCM4; Pharmacia). They contain the 35S promoter, the *Fed-1* leader sequence, and either 20 or 34 codons of *Fed-1* protein sequence fused in-frame to the *CAT* sequence at the BamHI site. Mutagenesis, transformation, regeneration, light treatments, and RNA gel blot analyses were performed as described in Methods. Induction ratios were measured as described in Methods and Figure 1. The horizontal line indicates the baseline at which no light response was observed.

DISCUSSION

Because normal responsiveness is restored when a nonsense codon at position 2 is replaced by either of two different sense codons, we concluded that the original nonsense mutation prevents the light response by preventing translation of *Fed-1* mRNA and not simply by altering its mRNA sequence. In addition, these data established that the particular amino acid encoded at this position is not critical for the response. The latter point distinguishes the *Fed-1* response from β-tubulin mRNA in mammalian cells (Cleveland and Yen, 1989; Theodorakis and Cleveland, 1992). In this situation, tubulin mRNA degradation is induced by tubulin subunits acting through a mechanism that recognizes the N-terminal region of the nascent peptide as it emerges from the ribosome, and mutations altering any of the four N-terminal amino acids block mRNA decay. In contrast, a leucine-for-alanine substitution at position 2 has little effect on the *Fed-1* system.

Inhibition of the light response by nonsense mutations and mutations preventing translational initiation provides strong circumstantial evidence that *Fed-1* mRNA must be translated for light to have its full effect on mRNA abundance. However, because most of these mutations do not entirely eliminate the light response, it is possible that some portion of the overall response occurs in the absence of translation, or, alternatively, the internal initiation sites (at positions 22 and 24) are active in the absence of the normal initiation site. Smeekens and collaborators (Smeekens et al., 1987, 1989) have shown that a truncated ferredoxin protein can be synthesized in vitro from an internal ATG in *Silene* ferredoxin mRNA. If internal initiation also occurs in vivo for *Fed-1* mRNA, the residual light response we observed for constructs such as MS1 and NS1 might reflect a low level of translation initiating at one or more of these internal ATGs. However, as discussed in the Results, these internal ATGs are not required for the light response when a functional *Fed-1* translation start site is present.

From a mechanistic point of view, it is important to determine whether nonsense mutations increase the level of *Fed-1* mRNA in the dark or decrease its abundance in the light. An increase would mean that untranslatable *Fed-1* mRNA is more stable than its wild-type counterpart, whereas a decrease would imply that mutant mRNA is destabilized. Precedents have been

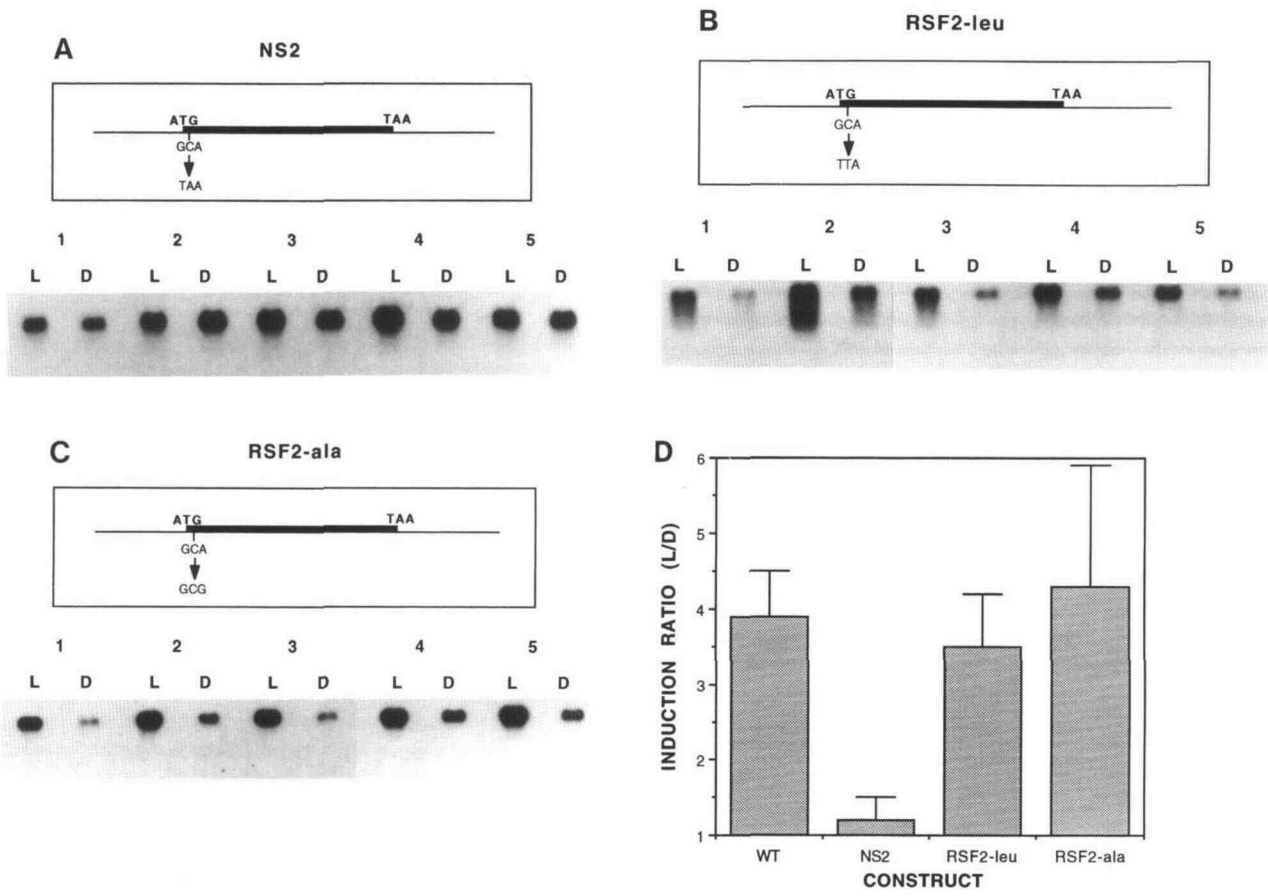

Figure 3. Gel Blots of RNA from Transgenic Plants Containing NS2 and Restored Reading Frame (RSF) Constructs.

The numbers above the autoradiographic images designate independent primary transformants, each of which is represented by clonally duplicated plants that were dark adapted and then either illuminated for 6 hr (lanes L) or allowed to remain in the dark prior to harvest (lanes D), as described in Methods.

(A) The NS2 construct was derived from the wild-type message construct (Elliott et al., 1989) by replacing the wild-type alanine (GCA) codon at position 2 with a nonsense codon (TAA).

(B) The RSF2-leu construct was prepared as was the NS2 construct, except that the alanine codon at position 2 was replaced with a leucine codon (TTA).

(C) The RSF2-ala construct was prepared as was the NS2 construct, except that the wild-type GCA codon at position 2 was replaced with a different alanine codon, GCG.

(D) Comparison of average induction ratios for NS2 and RSF2 constructs. Hybridization signals from the RNA gel blots shown in **(A)** to **(C)** were quantitated as described in Methods.

reported for yeast and mammals to support both possibilities, although examples of destabilization (nonsense-mediated decay) have been reported more frequently (for reviews, see Peltz and Jacobson, 1993; Sachs, 1993; Sullivan and Green, 1993). In plants, premature termination has been reported to reduce mRNA abundance, presumably by accelerating its decay (Jofuku et al., 1989; Vancanneyt et al., 1990; Voelker et al., 1990; Sullivan and Green, 1993).

Discrimination between these alternatives in the *Fed-1* system is complicated by the variability in absolute expression between different transgenic lines that is attributable to genomic position effects (Peach and Velten, 1991). However, the light-induced mRNA levels we observed for constructs with nonsense codons are usually lower than those for wild-type controls and seem to be generally comparable to the control levels after dark adaptation (L.F. Dickey and T.-T. Nguyen, unpublished data). Therefore, we believe it is more likely that blocking translation preferentially destabilizes *Fed-1* mRNA in the light; this is consistent with a model in which light-induced translation would stabilize the wild-type mRNA. This hypothesis makes several testable predictions. Wild-type *Fed-1* mRNA should be more efficiently translated, and its half life should be longer in illuminated plants as compared to dark controls. In addition, nontranslatable variants of *Fed-1* mRNA should

be less stable than the wild-type message in the light, while this difference should be reduced or eliminated in dark controls.

We do not yet know precisely how far translation must proceed into the *Fed-1* coding region to permit normal light regulation of the mRNA level. However, we have previously shown that light responsiveness is preserved when the *Fed-1* iLRE is fused to a firefly luciferase mRNA sequence, even though there is a stop codon in the luciferase leader sequence 45 bp downstream of the fusion site (Dickey et al., 1992b). Thus, it is likely that translation is required only at the 5′ end of the *Fed-1* mRNA. The *Fed-1* system may thus be compared to certain mammalian and yeast mRNAs whose abundance is sensitive to translational blocks early in the coding region but not to blocks near its 3′ end (Belgrader et al., 1993; Caponigro et al., 1993; Cheng and Maquat, 1993; Peltz et al., 1993). It has been argued (Belgrader et al., 1993) that export of these messages from the nucleus requires translation of the 5′ portion as it enters the cytoplasm, and it is possible that a similar mechanism could mediate the light effect on *Fed-1* mRNA. Testing this hypothesis will require testing constructs with nonsense codons at additional downstream positions as well as careful measurements of RNA accumulation in nuclear and cytoplasmic compartments.

Together with our earlier results (Elliott et al., 1989; Dickey et al., 1992b), the data presented here lead to an overall picture of *Fed-1* mRNA regulation by light that is remarkably similar to that emerging from recent work on iron regulation in cyanobacteria. Bovy et al. (1993b) reported that the half life of *Synechococcus* ferredoxin I mRNA is greater in the presence of abundant iron. Both the 5′ untranslated region and the first part of the coding region are required for this response (Bovy et al., 1993a), just as they are for the *Fed-1* light response (Dickey et al., 1992b). In addition, inhibitor experiments showed that the *Synechococcus* iron response depends on protein synthesis (Bovy et al., 1993a). This result is consistent with our observation that translation of the *Fed-1* message is required for light to affect its abundance in leaves of higher plants. Although many details remain to be discovered in both systems, it is noteworthy that the general features of this regulatory mechanism seem to have been conserved during the the evolution of higher plants and cyanobacteria.

METHODS

Constructs were derived from the Ferredoxin I (*Fed-1*) message construct (Elliott et al., 1989), which contains the entire *Fed-1* mRNA sequence under transcriptional control of a cauliflower mosaic virus (CaMV) 35S promoter derived originally from pBI121 (Jefferson et al., 1987). In all cases, polymerase chain reaction (PCR) was used to generate mutated clones. In the first PCR reaction, the wild-type message sequence in a pUC-derived plasmid was used as the template. Mutagenic primers contained the appropriate mutation flanked on either side by 10 nucleotides of wild-type sequence in the sense orientation. Each mutagenic primer was used together with the Stratagene M13 −20 primer to amplify a fragment containing the desired mutation. A second PCR reaction was performed using this mutated fragment as a primer together with the Stratagene M13 reverse primer to produce a fragment carrying the entire message sequence with the introduced mutation.

For *Fed::CAT* (chloramphenicol acetyltransferase) fusion constructs, the *CAT* gene was obtained from pCM4 (Pharmacia), and fusions were made using the PCR-based overlap extension technique (Ho et al., 1989). PCR reactions contained 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-Cl, pH 9, 0.1% Triton X-100, 0.2 mM deoxynucleotide triphosphates, 0.1 mM of each primer, 1 ng plasmid template, and 4.5 units of Taq DNA polymerase. Mineral oil (50 μL) was used to prevent evaporation. The thermal cycler was set for 2 min at 94°C, followed by annealing at 42°C for 2.5 min and polymerization at 72°C for 3 min. A total of 25 cycles was followed by a 7-min extension at 72°C. After cloning, each construct was sequenced to confirm the presence of the desired mutation and the absence of undesired alterations.

Mutagenized constructs were cloned into pBIN19 and transferred into *Agrobacterium tumefaciens* strain LBA4404 by triparental mating (Elliott et al., 1989). Plant transformation, regeneration, light treatments, and RNA analysis were all performed as previously described by Dickey et al. (1992b). Briefly, each primary transformant was divided during the regeneration process so that each transformation event was represented by a pair of clonally duplicated individual plants. These were transferred to soil and grown to mid-maturity (a height of 15 to 25 cm, with six leaves 4 to 8 cm long). At this point, all plants were dark adapted by placing them in total darkness at 22°C for 3 days. One member of each clonal duplicate pair was then returned to the light for 6 hr.

Total RNA was prepared from the two youngest leaves whose blades were completely unfolded. *Fed-1* mRNA was visualized by gel blot hybridization under conditions in which only transgene mRNA was detected, and hybridization signals were quantitated directly from the filters using a radioanalytic imaging system (AMBIS Systems, Inc., San Diego, CA). Light induction ratios were averaged from separate experiments on clonal duplicate pairs representing five to seven independent transformants.

ACKNOWLEDGMENTS

We acknowledge excellent technical assistance from J. Nolan Bryant and general laboratory support from Dolores A. Sowinski. Controlled environment plant growth space was provided by the Southeastern Plant Environment Laboratory (Raleigh, NC). We also thank Keith Everett for oligonucleotides and photographic services and Marie Petracek for comments on the manuscript. This project was supported by National Institutes of Health Grant No. GM43108 and funds from the North Carolina Agricultural Experiment Station.

Received April 18, 1994; accepted June 9, 1994.

REFERENCES

Belgrader, P., Cheng, J., and Maquat, L.E. (1993). Evidence to implicate translation by ribosomes in the mechanism by which

nonsense codons reduce the nuclear level of human triosephosphate isomerase mRNA. Proc. Natl. Acad. Sci. USA **90,** 482–486.

Bovy, A., de Kruif, J., de Vrieze, G., Borrias, M., and Weisbeek, P. (1993a). Iron-dependent protection of the *Synechococcus* ferredoxin I transcript against nucleolytic degradation requires *cis*-regulatory sequences in the 5′ part of the messenger RNA. Plant Mol. Biol. **22,** 1047–1065.

Bovy, A., de Vrieze, G., Lugones, L., van Horssen, P., van den Berg, C., Borrias, M., and Weisbeek, P. (1993b). Iron-dependent stability of the ferredoxin I transcripts from the cyanobacterial strains *Synechococcus* species PCC 7942 and *Anabena* species PCC 7937. Mol. Microbiol. **7,** 429–439.

Brewer, G., and Ross, J. (1990). Messenger RNA turnover in cell-free extracts. Methods Enzymol. **181,** 202–209.

Byrne, D.H., Seeley, K.A., and Colbert, J.T. (1993). Half lives of oat mRNA in vivo and in a polysome-based in vitro system. Planta **189,** 249–256.

Caponigro, G., Muhlrad, D., and Parker, R. (1993). A small segment of the MATα1 transcript promotes mRNA decay in *Saccharomyces cerevisiae*: A stimulatory role for rare codons. Mol. Cell. Biol. **13,** 5141–5148.

Cheng, J., and Maquat, L.E. (1993). Nonsense codons can reduce the abundance of nuclear mRNA without affecting the abundance of pre-mRNA or the half life of cytoplasmic mRNA. Mol. Cell. Biol. **13,** 1892–1902.

Cleveland, D.W., and Yen, T.J. (1989). Multiple determinants of eukaryotic mRNA stability. New Biol. **1,** 121–126.

Dickey, L.F., Gallo-Meagher, M., and Thompson, W.F. (1992a). Ferredoxin gene expression and its regulation by light. In Control of Gene Expression, D.P.S. Verma, ed (Boca Raton, FL: CRC Press, Inc.), pp. 221–222.

Dickey, L.F., Gallo-Meagher, M., and Thompson, W.F. (1992b). Light regulatory sequences are located within the 5′ portion of the *Fed-1* message sequence. EMBO J. **11,** 2311–2317.

Dobres, M.S., Elliott, R.C., Watson, J.C., and Thompson, W.F. (1987). A phytochrome-regulated pea transcript encodes ferredoxin I. Plant Mol. Biol. **8,** 53–59.

Elliott, R.C., Dickey, L.F., White, M.J., and Thompson, W.F. (1989). *cis*-Acting elements for light regulation of pea ferredoxin I gene expression are located within transcribed sequences. Plant Cell **1,** 691–698.

Gallie, D.R. (1993). Posttranscriptional regulation of gene expression in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **44,** 77–105.

Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K., and Pease, L.R. (1989). Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene **77,** 51–59.

Jefferson, R.A., Kavanagh, T.A., and Bevan, M.W. (1987). GUS fusions: β-Glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. **6,** 3901–3907.

Jofuku, K.D., Schipper, R.D., and Goldberg, R.B. (1989). A frameshift mutation prevents Kunitz trypsin inhibitor mRNA accumulation in soybean embryos. Plant Cell **1,** 427–435.

Kaufman, L.S., Roberts, L.R., Briggs, W.R., and Thompson, W.F. (1986). Phytochrome control of specific mRNA levels in developing pea buds. Kinetics of accumulation, reciprocity, and escape kinetics of the low fluence response. Plant Physiol. **81,** 1033–1038.

Kuhlemeier, C. (1992). Transcriptional and post-transcriptional regulation of gene expression in plants. Plant Mol. Biol. **19,** 1–14.

Neuhaus, G., Bowler, C., Kern, R., and Chua, N.-H. (1993). Calcium/calmodulin-dependent and -independent phytochrome signal transduction pathways. Cell **73,** 937–952.

Peach, C., and Velten, J. (1991). Transgene expression variability (position effect) of CAT and GUS reporter genes driven by linked divergent T-DNA promoters. Plant Mol. Biol. **17,** 49–60.

Peltz, S.W., and Jacobson, A. (1993). mRNA turnover in *Saccharomyces cerevisiae*. In Control of Messenger RNA Stability, G. Brawerman and J. Belasco, eds (New York: Academic Press), pp. 291–328.

Peltz, S.W., Brown, A.H., and Jacobson, A. (1993). mRNA destabilization triggered by premature translational termination depends on at least three *cis*-acting sequence elements and one *trans*-acting factor. Genes Dev. **7,** 1737–1754.

Sachs, A.B. (1993). Messenger RNA degradation in eukaryotes. Cell **74,** 413–421.

Smeekens, S., van Steeg, H., Bauerle, C., Bettenbroek, H., Keegstra, K., and Weisbeek, P. (1987). Import into chloroplasts of a yeast mitochondrial protein directed by ferredoxin and plastocyanin transit peptides. Plant Mol. Biol. **9,** 377–388.

Smeekens, S., Geerts, D., Bauerle, C., and Weisbeek, P. (1989). Essential function in chloroplast recognition of the ferredoxin transit peptide processing region. Mol. Gen. Genet. **216,** 178–182.

Sullivan, M.L., and Green, P.J. (1993). Post-transcriptional regulation of nuclear-encoded genes in higher plants: The roles of mRNA stability and translation. Plant Mol. Biol. **23,** 1091–1104.

Tanzer, M.M., and Meagher, R.B. (1994). Faithful degradation of soybean *rbcS* mRNA in vitro. Mol. Cell. Biol. **14,** 2640–2650.

Theodorakis, N.G., and Cleveland, D.W. (1992). Physical evidence for cotranslational regulation of β-tubulin mRNA degradation. Mol. Cell. Biol. **12,** 791–799.

Thompson, W.F. (1988). Photoregulation: Diverse gene responses in greening seedlings. Plant Cell Environ. **11,** 319–328.

Thompson, W.F., and White, M.J. (1991). Physiological and molecular studies of light-regulated nuclear genes in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42,** 423–466.

Vancanneyt, G., Rosahl, S., and Willmitzer, L. (1990). Translatability of a plant mRNA strongly influences its accumulation in transgenic plants. Nucl. Acids Res. **18,** 2917–2921.

Voelker, T.A., Moreno, J., and Chrispeels, M.J. (1990). Expression analysis of a pseudogene in transgenic tobacco: A frameshift mutation prevents mRNA accumulation. Plant Cell **2,** 255–261.

Vorst, O., van Dam, F., Weisbeek, P., and Smeekens, S. (1993). Light-regulated expression of the *Arabidopsis thaliana ferredoxin A* gene involves both transcriptional and post-transcriptional processes. Plant J. **3,** 793–803.

Wada, K., Aota, S., Tsuchiya, R., Ishibashi, F., Gojobori, T., and Ikemura, T. (1992). Codon usage tabulated from the GenBank genetic sequence data. Nucl. Acids Res. **20**(Suppl.), 2111–2118.